Karyotype and C-Banding Patterns of Mitotic Chromosomes in Diploid Bromegrass (*Bromus riparius* Rehm)

Metin Tuna, Kulvinder S. Gill, and Kenneth P. Vogel*

ABSTRACT

Previous cytogenetic studies of the genus *Bromus* L. were limited to chromosome counts and construction of karyotypes on the basis of Feulgen staining. Since the chromosomes of *Bromus* are similar in morphology, these karyotypes are of limited use for chromosome identification and genome analysis. The objectives of this study were to develop and evaluate a Giemsa C-banding procedure to use in identification of individual bromegrass chromosomes and to develop a karyotype for diploid *Bromus riparius* Rehm. ($2n = 14$; PI 440215). All chromosomes had one or more C-bands which were located mainly at telomeric regions. A group (I) of four pairs of chromosomes had telomeric bands on only one arm and could be differentiated. In this group, one pair had an interstitial C-band along with a telomeric band, one pair had a nucleolus organizer region (NOR) at a subtelomeric location on the short arm, and the other two pair could be distinguished by centromere location. The other group (II) of three pairs of chromosomes had telomeric bands on both arms. The unequivocal identification of specific chromosomes of Group II was not possible in all cells because of their similarity and differential condensation of chromosomes. Chromosomes of both groups were either metacentric or submetacentric. The total length of individual chromosomes ranged from 5.58 to 6.87 μm and the arm ratios ranged from 1.02 to 1.5. The homologous chromosomes were paired and assigned numbers I to VII in decreasing length. A karyotype was constructed by means of the C-bands, mean chromosome lengths, and arm ratios. The C-banding procedure used in this study could be used to developed karyotypes for the other species of the genus *Bromus* and these C-banded karyotypes could be used to compare genomes within the genus.

THE GENUS *BROMUS* CONTAINS more than 100 species distributed over all continents (Gould and Shaw, 1983). The ploidy level within the genus varies from diploid to decaploid (Armstrong, 1991). Feulgen staining based karyotypes have been constructed for some species of the genus *Bromus* (Rychlewski, 1970; Armstrong, 1977; Stebbins, 1981). It has been difficult to identify all of the chromosomes because the chromosomes are similar in morphology. Genetic relationships among a few species have been studied by means of crossability and chromosome pairing data (Stebbins, 1981; Armstrong, 1991). Giemsa C-banding technique, which stains constitutive heterochromatin, is a technique that has been used successfully in many species to identify individual chromosomes and to establish genomic relationships among species (Vosa, 1975; Cai and Chinnappa, 1987; Fominaya et al., 1988; Gill and Sears, 1988; Tayyar et al., 1994; Falistocco et al., 1995). The C-banding procedure has not been explored fully for chromosome identification and genome analysis in *Bromus*.

Bromus inermis L. ($2n = 56$), or smooth bromegrass, is one of the most widely used forage species in agriculture. Progenitor species of this complex polyploid have not been fully delineated (Vogel et al., 1996). In this report, we describe an effective C-banding procedure for bromegrasses that was used to identify chromosomes and construct a karyotype of a possible progenitor, the diploid species (*B. riparius* Rehm.), of the *B. inermis* complex. The karyotype is based on chromosome length, arm ratio, and C-banding patterns.

The *B. riparius* accession used in this study, PI 440215 (National Plant Germplasm System, 1999; Armstrong, 1987), was collected from Chimkent in Kazakhstan in 1977. Armstrong (1987) determined its ploidy level as $2n = 14$ and suggested that it could be a progenitor of the *Bromus inermis* complex since its morphology resembles that of the tetraploid forms of *B. inermis* collected from the same region. It is the only diploid *B. riparius* in the USDA Plant Germplasm System that we have identified to date.

MATERIALS AND METHOD

Seeds of diploid *Bromus riparius* Rehm. (PI 440215) were obtained from the USDA Regional Plant Introduction Station, Pullman, WA. Seeds were used to grow plants in the greenhouse and to produce seedlings in germination boxes. Twenty plants were grown in pots filled a mixture of soil, Perlite, and peat moss (2:1:1 v/v/v). Plants were exposed to 16-h photoperiod during the period when root tips were collected. They were maintained in a vegetative stage by clipping. Actively growing root tips were collected from potted plants 3 to 4 wk after each clipping. Seeds were germinated in germination boxes containing germination paper saturated with distilled water. Imbibed seeds were kept at room temperature for 1 d before they were transferred to a refrigerator at 0 to 4°C for one to a few days (until the majority of seeds appeared to be germinating). Boxes were then placed in the dark at room temperature and fast growing root tips were collected when they reached 1 to 1.5 cm in length. Harvested root tips from potted plants or germinated seeds were placed in vials containing 0.05% colchicine (w/v). The colchicine solution was drained from the vials after 1 to 1.5 h and replaced with a fixative of ethanol: glacial acetic acid (3:1, v/v) for 2 wk to a few months.

The C-banding method used was that described by Giraldez et al. (1979) and slightly modified as follows. Root tips were

M. Tuna, Dep. of Agronomy, Tekirdag Agriculture Faculty, Univ. of Trakya, Tekirdag, Turkey; K.S. Gill, Dep. of Agronomy, Univ. of Nebraska, Lincoln, NE 68583-0915; K.P. Vogel, USDA-ARS, Wheat, Sorghum, and Forage Res. Unit, 344 Keim Hall, Univ. of Nebraska, P.O. Box 830937, Lincoln, NE 68507-0937. Contribution from Nebraska Agric. Res. Div., Journal Series No. 12 973. Reported research is from a dissertation submitted by the senior author (Metin Tuna) in partial fulfillment of the requirements for the Ph.D. degree at the University of Nebraska. Received 3 April 2000. *Corresponding author (kpv@unlserve.unl.edu).

Published in Crop Sci. 41:831–834 (2001).

Abbreviations: Mb, megabases; NOR, nucleolus organizer region; Chr, chromosome; Sat, satellite.

stained with 1% acetocarmine (1 g acetocarmine per 100 mL of 45% acetic acid) for about 30 min before making preparations by the squash technique. Slides were examined under the microscope and those which exhibited a relatively high mitotic index were quickly frozen by placing them in a −80°C freezer for at least 2 h. Cover slips were then removed and slides were placed in 95% (v/v) ethanol at room temperature, overnight. After dehydration, slides were air dried at room temperature for 3 to 4 h, subsequently incubated for 3 min in 0.2 *M* HCl at 60°C in a water bath, and washed briefly in distilled water. Slides then were placed in saturated $Ba(OH)_2$ solution at room temperature for 8 min, washed carefully in distilled water until all the barium crystals were removed, and then placed in 2 × SSC solution (1× is 0.15 *M* NaCl plus 0.015 *M* citric acid) at 60°C for 1 h before transferring them to the staining solution containing 4% solution of Giemsa stain (v/v) in phosphate buffer for 12 min. Phosphate buffer was comprised of 62% 0.07 *M* Na_2HPO_4 and 38% 0.07 *M* KH_2PO_4 solutions. After staining, slides were quickly rinsed in distilled water and dried for several hours. For observations slides were mounted in Permount.

Cells with well-spread chromosomes were identified and an image of each cell was captured by a Spot I digital camera (Diagnostic Instruments Inc., Sterling Heights, MI)[1]. Enlarged pictures (3000 ×) of 13 cells with well-spread metaphase chromosomes and many other cells from a total of 17 different plants were used for analysis and construction of karyotypes. Chromosome measurements were made on the enlarged prints and converted to micrometers by relating measurements from enlarged prints with measurements made in a microscope with a micrometer. The chromosomes were identified on the basis of their total length, arm length ratio (long/short arm), and C-banding patterns. Chromosomes in the karyotype were arranged into groups of two according to homology, following decreasing mean chromosome length, with the exception of the pair with a NOR which was placed at the end of the karyotype.

[1] Names of products are included for the benefit of the reader and do not imply endorsement by USDA or the University of Nebraska.

RESULTS AND DISCUSSION

C-banded mitotic metaphase chromosomes of diploid *B. riparius* are shown in Fig. 1 and a detailed C-banded karyotype is presented in Fig. 2 with accompanying total lengths and arm length ratios in Table 1. Standard deviations also were calculated for each chromosome (Table 1). Different contraction of chromosomes among cells used to develop the karyotype was the main reason for the high standard deviation value. Within the chromosome complement, six pairs of chromosomes were metacentric (chromosomes I, II, III, IV, V, and VII) and one pair is submetacentric (chromosome VI) although the arm ratio means ranged from 1.02 to 1.5. Our results with respect to the location of centromeres in chromosomes are in agreement with previous reports (Armstrong, 1991). One pair of chromosomes had large satellites at a subtelomeric location of the short arm. The length of the secondary constriction varied among cells.

The total haploid genome length was determined as 42.89 μm and contained 3.07 pg/1C DNA (Tuna et al. 2000, unpublished results) which is approximately 3000 megabases (Mb). The genome size is slightly larger than maize (2500 Mb) but is roughly five times smaller than hexaploid wheat (16 000 Mb) (Arumuganathan and Earle, 1991). The length of chromosomes ranged from 5.58 to 6.87 μm. The mean total length of chromosome I was the longest but not in all cells. Chromosome I was the longest chromosome in 8 out of 13 cells and chromosomes II, III, and IV were the longest chromo-

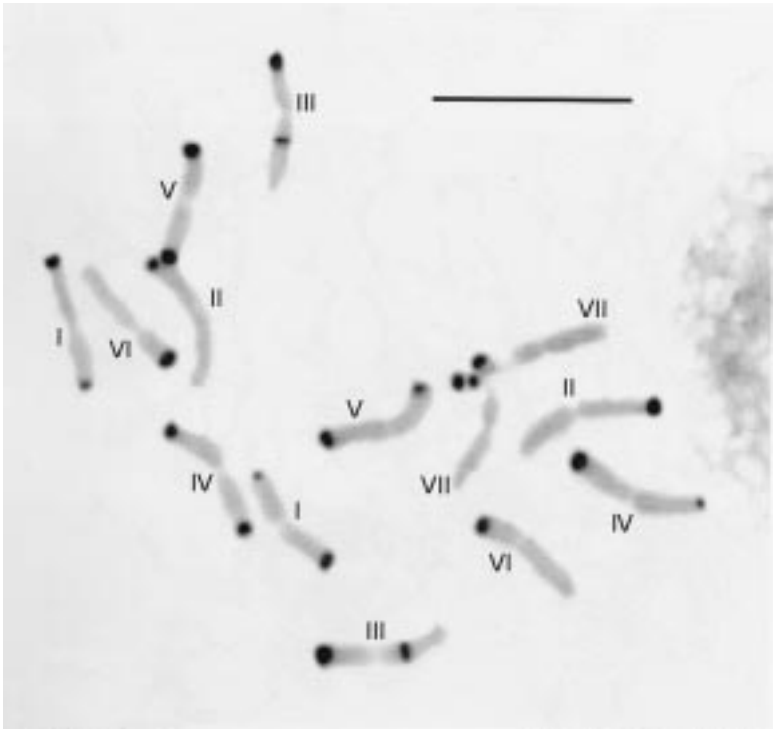

Fig. 1. C-banded mitotic metaphase chromosomes of *B. riparius* (PI 440215, 2*n* = 14). Bar = 10 μm.

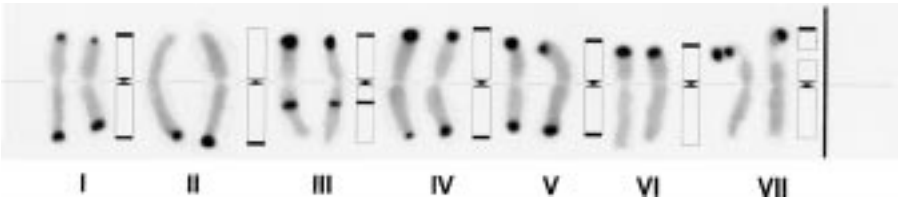

Fig. 2. C-banded karyotype of diploid *B. riparius* (PI 440215, 2*n* = 14) based on the cell presented in Fig. 1. Bar = 10 μm.

some in 2, 2, and 1 cell, respectively. Chromosome VI was the shortest chromosome in 8 cells out of 13 while chromosome V was the shortest chromosome in the other 5 cells.

Three pairs of chromosomes showed telomeric bands on both arms, whereas the other four pairs showed a telomeric band on only one arm. Occasionally, a C-band was observed at the NOR site of one or both chromosomes of pair VII depending upon the cell. Constitutive heterochromatin was located mainly at telomeric regions in the diploid *B. riparius*, as all the chromosomes had major telomeric C-bands. This characteristic of the heterochromatin is similar to that of rye, *Secale cereale* L., (Giraldez et al., 1979), which also shows mainly telomeric C-bands.

Although C-banding patterns often show a high degree of polymorphism in cross-pollinated species (Gill, 1981; Endo and Gill, 1984; Bauchan and Hossain, 1999), all the observed bands were quite consistent in plants of PI 440215 studied here except for the telomeric band on the short arm of chromosome II. This band was absent in 10 of the 17 plants of the accession. Furthermore, one plant was heterozygous for this band. In this plant, one of the chromosomes II had a telomeric band only on the long arm, while its homolog had a telomeric band on both arms. C-band polymorphism and heterozygosity was also reported in *Allium* (Cai and Chinnappa, 1987); alfalfa (*Medicago sativa* L.) (Massoud et al., 1991); timothy (*Phleum pratense* L.) (Cai and Bullen, 1991), and Russian wildrye [*Psathyrostachys juncea* (Fischer) Nevski] (Wei et al., 1995).

The banding patterns and morphology of the seven somatic chromosomes are described as follows.

1. Chromosome I was the longest chromosome (6.87 μm) with telomeric bands in both arms. It had a median centromere with an arm ratio of 1.14.
2. Chromosome II had a total length of 6.62 μm, a median centromere with an arm ratio of 1.07, and a telomeric band in only one arm. This was the only chromosome in which a C-banding polymorphism was observed.
3. Chromosome III had a total length of 6.37 μm, a median centromere with an arm ratio of 1.18, a telomeric band on the short arm, and one interstitial band in the long arm. This was the most easily identifiable chromosome because of this interstitial band.
4. Chromosome IV had a total length of 5.98 μm, a median centromere with an arm ratio of 1.05, and telomeric bands present on both arms.
5. Chromosome V also had a median centromere with an arm ratio of 1.10, and telomeric bands on both arms. It was shorter than chromosome IV with a total length of 5.65 μm.
6. Chromosome VI was the shortest chromosome with a total length of 5.58 μm. It had a submedian centromere with an arm ratio of 1.5, and a telomeric band on only the short arm.
7. Chromosome VII was a satellite chromosome with a total length of 5.82 μm and a median centromere. The arm ratio was 1.02 and it had a telomeric band on the satellite arm.

On the basis of chromosome length, arm ratio and C-banding patterns, the chromosomes of *B. riparius* (PI 440215) can be divided into two groups on the basis of their C-banding patterns. Group I consists of four chromosome pairs (chromosome II, III, VI, and VII) with one telomeric band on either the short or long arm, while group II is comprised of 3 chromosome pairs (chromosome I, IV, and V) with two telomeric bands.

The chromosomes of group I could be identified from each other very easily. Chromosome III had a very distinctive interstitial band, which allowed it to be differentiated from the other chromosomes of group I. Chromosomes II and VII were quite similar in size, arm ratio, and banding pattern, but chromosome VII had a NOR

Table 1. The chromosomes of the diploid *Bromus riparius* based on 13 cells.

Chr.	Long arm Mean SD§	Sort arm Mean SD§	Total length Mean SD§	Sat. Size Mean SD§	Arm Ratio‡ Mean SD§	Chr. Type
	μm					
I	3.68 ± 0.71	3.19 ± 0.59	6.87 ± 1.28		1.14 ± 0.07	median‡
II	3.41 ± 0.64	3.18 ± 0.55	6.62 ± 1.18		1.07 ± 0.04	median
III	3.48 ± 0.56	2.95 ± 0.43	6.37 ± 0.97		1.18 ± 0.06	median
IV	3.31 ± 0.40	3.13 ± 0.35	5.98 ± 0.88		1.05 ± 0.04	median
V	2.96 ± 0.36	2.69 ± 0.39	5.65 ± 0.75		1.10 ± 0.04	median
VI	3.35 ± 0.67	2.24 ± 0.48	5.58 ± 1.14		1.50 ± 0.12	submedian¶
VII	2.83 ± 0.44	1.43 ± 0.23	5.82 ± 1.06	1.48 ± 0.55	1.02 ± 0.41	satellite

† Arm ratio = Length of the long arm/length of the short arm.
‡ median = Arm ratio is lower than 1.50.
¶ submedian = Arm ratio is higher than 1.50.
§ SD = Standard deviation.

on the short arm. Chromosome VI also was identifiable from the other chromosomes of the group I because of its submedian centromere.

The chromosomes of group II with their similar morphology and banding patterns could not be unequivocally identified from each other in all cells because of differences in chromosome spreads among cells. However, their mean arm length ratio and total length were useful to distinguish chromosomes in this group. Chromosomes I and IV were similar in morphology but they had different arm ratios and total lengths. Chromosome I was the longest chromosome while chromosome V was the shortest chromosome after chromosome VI. Chromosomes IV and V were the most similar to each other of any chromosomes within the karyotype. Therefore, it was difficult to differentiate them from each other. Fortunately, their length was slightly different but these two chromosomes still can be confused easily if there is difference in condensation.

The C-banding technique was successfully used to identify chromosomes of diploid *B. riparius* and it should be a useful method to compare species relationships within the genus *Bromus*. C-banding analysis on several other related species including tetraploid and octaploid *B. inermis* are in progress for the genus.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. George Graef, Assoc. Professor, Department of Agronomy, University of Nebraska, Dr. Paul Staswick, Professor, Department of Agronomy, University of Nebraska, Dr. Georgia Eizenga, Research Geneticist, USDA-ARS, Stuttgart, AR, Dr. Richard Wang, Research Geneticist, USDA-ARS, Logan, UT, and Dr. Gary R. Bauchan, Research Geneticist, USDA-ARS, Beltsville, MD, for their reviews and valuable suggestions on the manuscript. The authors also thank the Turkish government for supporting the principal author to conduct this research as a part of his Ph. D. dissertation.

REFERENCES

Armstrong, K.C. 1977. Karyotypic models for the A and B genomes of *Bromus inermis*. Z. Pflanzenzücht 78:244–252.

Armstrong, K.C. 1987. Chromosome numbers of perennial *Bromus* species collected in the USSR. Can. J. Plant Sci. 67:267–269.

Armstrong, K.C. 1991. Chromosome evolution in *Bromus*. p. 363–377. *In* T. Tsuchiya and T.K. Gupta (ed.) Chromosome engineering in plants: Genetics, breeding, evolution. Part B. Elsevier, Amsterdam, the Netherlands.

Arumuganathan, K. and E.D. Earle. 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. 9:208–218.

Bauchan, G.R., and M.A. Hossain. 1999. Constitutive heterochromatin DNA polymorphisms in diploid *Medicago sativa* ssp. *falcata*. Genome 42:930–935.

Cai, Q., and C.C. Chinnappa. 1987. Giemsa C-banded karyotypes of seven North American species of *Allium*. Am. J. Bot. 74:1087–1092.

Cai, Q., and M.R. Bullen. 1991. Characterization of genomes of timothy (*Phleum pratense* L.): I. Karyotypes and C-banding patterns in cultivated timothy and two wild relatives. Genome 34:52–58.

Endo, T.R., and B.S. Gill. 1984. The heterochromatin distribution and genome evolution in diploid species of *Elymus* and *Agropyron*. Can. J. Genet. Cytol. 26:669–678.

Falistocco, E., M. Falcinelli, and F. Veronesi. 1995. Karyotype and C-banding pattern of mitotic chromosomes in alfalfa, *Medicago sativa* L. Plant Breed. 114:451–453.

Fominaya, A., C. Vega, and E. Ferrer. 1988. Giemsa C-banded karyotypes of *Avena* species. Genome 30:627–632.

Gill, B.S. 1981. Evolutionary relationships based on heterochromatin bands in six species of the *Triticeae*. J. Hered. 72:391–394.

Gill, B.S., and R.G. Sears. 1988. The current status of chromosome analysis in wheat. p. 299–321. *In* J.P. Gustafson and R. Appels (ed.) Chromosome structure and function. Plenum, New York.

Giraldez, R., M.C. Cermeno, and J. Orellana. 1979. Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye. Z. Pflanzenzücht 83:40–48.

Gould, F.W., and R.B. Shaw. 1983. Grass systematics. 2nd ed. Texas A&M Univ. Press, College Station, TX.

Masoud, S.A., B.S. Gill, and L.B. Johnson. 1991. C-banding of alfalfa chromosomes: Standard karyotype and analysis of a somaclonal variant. J. Hered. 82:335–338.

National Plant Germplasm System, U.S. Department of Agriculture, 1999. (http://www.ars-grin.gov/npgs/; verified January 5, 2001).

Rychlewski, J. 1970. Karyology of species of the genus Bromus L. Acta Biol. Cracov. (Ser. Bot.) 13:23–35.

Stebbins, G.L. 1981. Chromosomes and evolution in the genus *Bromus*. Bot. Jahr. System. Pflanzengescher Pflanzengeog 102:359–379.

Tayyar, R.I., A.J. Lukaszewski, and J.G. Waines. 1994. Chromosome banding patterns in the annual species of *Cicer*. Genome 37:656–663.

Vosa, C.G. 1975. The use of Giemsa and other staining techniques in karyotype analysis. Curr Adv Plant Sci. 14:495–510.

Vogel, K.P., K.J. Moore, and L.W. Moser. 1996. Bromegrasses. p. 535–567. *In* L.E. Moser et al. (ed.) Cool-season forage grasses. Agron. Monogr. 34, ASA, CSSA, SSSA, Madison, WI.

Wei, J.Z., W.F. Campbell, and R.R-C. Wang. 1995. Standard Giemsa C-banded karyotype of Russian wildrye (*Psathyrostachys juncea*) and its use in identification of a deletion-translocation heterozygote. Genome 38:1262–1270.